Some Observations on Acinetaria.

By

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With Plates VII and VIII.

Part I.—The "Tinctin-körper" of Acinetaria and the Conjugation of Acineta papillifera.

Contents.

I. Introductory.

The observations described in this paper were begun on a culture of Tokophrya elongata, which was given to me at Munich by Professor Hertwig in the summer semester of 1902. This culture was followed for a period of about six months, when it was unfortunately lost, but as during this time I had never seen any indication of conjugation, I thought it better to place the results I had obtained on one side until the occurrence of a more favourable opportunity.

During the early summer of 1907, whilst working in Norfolk, I found an Acinetarian which corresponded with Keppen's Acineta papillifera, in great abundance on the stems of Cordylophora lacustris in Hickling Broad. During the early part of September an epidemic of conjugation set in, and although, owing to the pressure of other duties, I was not able to follow the stages in the living form as completely as I should have wished, I was able to obtain enough fixed material to work through the main nuclear changes in this process. In the second part of this paper the life-history of a new Acinetarian parasite Tachyblaston ephelotensis is described, and in a future paper I hope to deal more fully with some general questions as regards the mechanism of the tentacles, as well as the relations between the lageniform and proboscidiform individual in that remarkable animal Ophryodendron.

I should like to take this opportunity of thanking Professor Hertwig, to whom the inception of this work is entirely due, for the great kindness he showed me at Munich, and Professor Graham Kerr for allowing me to work through my material in the laboratory at Glasgow University.

II. The "Tinctin-körper" of Acinetaria with some Observations upon the Nuclear Changes of Tokophrya elongata.

Before proceeding to give an account of the conjugation of Acineta papillifera, it will be necessary to give some account of those isolated masses of chromatin, the "Tinctin-körper" of Plate, which may occur in all free-living Acinetarians, and which enormously increase the difficulties of work upon the nuclear changes during conjugation.

Plate in 1886 first drew attention to certain rounded bodies occurring in the cytoplasm of Dendrocometes which were not blackened by osmic acid, and which were stained by safranin, and also, though less brilliantly, by carmine.

He called these bodies "Tinctin-körper," and noted that in some individuals they seem to be altogether absent.

When present they are usually more or less rounded bodies measuring up to ·006 mm., but occasionally they assume "eine wurst-formige unregelmässig längliche Gestalt und erreichen dann auch eine viel betractlichere Grosse."

At first he was inclined to believe that these bodies were equivalent to the micronuclei of the Ciliates, but he concludes by stating that the present state of our knowledge "lässt es rathsamer erscheinen sie vor der Hand nur als eigenartige Produkte des Stoffwechsels anzusehen."

Schneider also observed these structures in S t y l o c o m e t e s, but was inclined to believe that the "Tinctin-körper" are the products of the degeneration of the old macronucleus after conjugation.

This view is also upheld by Bütschli (in 1889) "Ich halte diese Ansicht fur recht wahrscheinlich um so mehr, als wir ja auch bei den Ciliaten erfuhren, dass die Fragmente des Alten Ma. N. haüfig sehr lange erhalten bleiben, und bei der Theilung auf die Nachkommen ubergehen können, wie es für die Tinctinkorper der Dendrocometinen gilt."

Sand (1901) in his monograph puts forward the view (p. 31) that "lorsqù'un Acinètien suce une proie, le sarcode de celle-ci, dès son entrée dans le cytoplasma du Tentaculifère s'amasse en sphères de mêmes dimensions que les sphères de tinctine; comme le cytoplasma mort est très colorable, et que du reste, les sphères contiennent, outre le cytoplasma le noyau de la proie, elles se colorent fortement par les teintures nucléaires."

Personally I believe that under the name of "Tinctin-körper" chromatic granules which may originate in one of three distinct ways are confused—those derived

(1) From the ingested nucleus of the prey,

(2) From the degenerating macronucleus after conjugation,

(3) Possibly to a very slight extent in some cases from

fragments of the macronucleus thrown out in connection with the formation of a digestive ferment.

Of these three modes of origin the first is by far the most common since conjugating Acinetaria are relatively quite rare. But it would still seem necessary to retain the general term "Tinctin-körper" since practically these bodies are only distinguishable when the details of the past life of the individual under examination are known.

Tokophrya (Bütschli) elongata (Clap. et Lachm.) is a more or less cylindrical Acinetan provided with a short stalk and with tentacles scattered in rather indefinite groups over the surface of the body.

It is an exceedingly easy form to cultivate, as it feeds readily upon almost all Ciliates, and appears to have few enemies.

My cultures were kept in watch-glasses, to the bottoms of which the young Acinetarians fixed themselves by their stalks; they were generally fed upon Stentor, but when the supply of Stentor was short they seemed to thrive equally well on Paramecia.

When the Stentor came in contact with the tentacles of the Acinetan, after some short struggles the cilia of the former ceased to beat and the animal remained paralysed.

As the Acinetan was very much smaller than its prey the first attack was rarely fatal, but if, as generally happened in a healthy culture, a single Stentor was attacked by five or six Acinetarians the whole animal would disappear at the end of a couple of hours.

To the details of this process and the mechanism of the tentacles I hope to return in a future paper in which I wish to describe certain feeding experiments made on Ephelota gemmipara.

Tokophrya elongata reproduces itself by the formation of internal hypotrichous ciliated buds, which escape through "the birth opening"—a lateral slit in the pellicule.

In a normal Tokophrya elongata the macronucleus is a band-shaped structure passing down the long axis of the body, but in addition to this there are always scattered in the cytoplasm a number of granules of chromatin, the "Tinctin-körper."

There seem to me to be three lines of evidence showing that the ordinary "Tinctin-körper" cannot be regarded as integral parts of a fragmented macronucleus—

(1) From their behaviour during reproduction and conjugation.

(2) From their behaviour during starvation and feeding.

(3) From their absence in parasitic forms such as Tachyblaston ephelotensis to be described later.

(1) There is no trace of a connecting thread between the "Tinctin-körper" such as is present in the macronuclei of Stentor and Holophrya, and there is no reunion to a single mass previous to division, such as has been described for some Hypotrichous Ciliates with a fragmented macronucleus.

In dividing Tokophrya the macronucleus becomes twisted upon itself in the region of the future bud, but in all the stages of division isolated "Tinctin-körper" are to be found both in the cytoplasm of the mother and of the bud (Pl. VIII, fig. 2).

In conjugating Acineta papillifera at stages at which the macronucleus was still intact individuals were found in which the "Tinctin-körper" seemed to be collected in the lower part of the theca, quite apart from the macronucleus.

(2) Their Behaviour during Starvation and Feeding.—During starvation there is a tendency for the "Tinctin-körper" to disappear, though even in the most complete cases (Pl. VIII, figs. 3 and 4) some remains were present. In Pl. VIII, fig. 4, which represented the last stage of starvation, the individual measured $25\,\mu$ in length and $18\,\mu$ in breadth, a normal well-fed Tokophrya from the parent culture measuring $143\,\mu$ by $58\,\mu$. The tentacles and most of the cytoplasm had disappeared, but the nucleus seemed quite healthy staining rather more readily than in the well-fed forms.

In preparations made a quarter and half an hour after feeding slightly-starved Tokophrya upon Stentor the "Tinctin-körper" showed no marked increase over the control form, and it is interesting to note that up to this stage the nuclei of the Stentor, although slightly swollen, had not been ingested. But in individuals which had ingested the nuclei of the prey enormous masses of "Tinctin-körper" were found resulting in such an appearance as is figured in Pl. VIII, fig. 1, in which the whole of the cytoplasm is blocked with "Tinctin-körper."

It is remarkable how little attention has been hitherto paid to the possible presence of ingested chromatin in Protozoa, although it is evident that unless the process of digestion is extremely rapid, these ingested masses may quite easily form a considerable source of error in the description of the nucleus of any holozoic protozoon.

As far as I am aware the only careful description of the appearance and behaviour of ingested chromatin in a Protozoon is to be found in Schaudinn's account of Trichosphærium sieboldii. Schaudinn (p. 81) found that in Trichosphærium the nuclei eight hours after ingestion were not greatly changed; in later stages the linin is dissolved, and the chromatin sinks to the lower side of the nucleus. "Das Chromatin wird nun auch allmählich gelöst, und nimmt hierbei meist Kugelgestalt an. Es schien mir, als ob hierbei seine Farbbarkeit zunimmt was vielleicht darauf beruht, dass bei der Verdauung ein nicht färbbarer Theil seiner substanz früher gelöst wird, während die färbbarer Theilchen dichter zusammengedrängt werden und daher in ihrer Gesammtheit dunkler gefärbt erscheinen."

As regards the details of this process of digestion in Acinetaria, the earlier stages are passed through whilst the nucleus is still lying in the cytoplasm of its prey. But in the early stages of the degeneration of the macronucleus of a conjugating form quite analogous early stages of digestion are to be found in the cytoplasm of the Acinetarian.

The later stages of the digestion as figured by Schaudinn have an absolutely identical appearance to the "Tinctin-körper" of the Acinetaria.

(3) In a parasitic form, Tachyblaston ephelotensis, which I describe in the second part of this paper, I could never find any trace of "Tinctin-körper."

It is interesting to note that in this case the nucleus of the host was never ingested by the parasite.

From a consideration of these points it will, I think, become evident that, in the great majority of cases, the scattered chromatin granules found in Acinetaria can only be regarded as the partially digested nucleus of their prey.

III. The Conjugation of Acineta papillifera.

Without attempting to give a history of our knowledge of conjugation in the Acinetaria (which has already been fully done by Bütschli in his great work on Protozoa as regards the earlier period), it will be necessary to give a short account of the work done by later observers of this process, and more particularly of Keppen's work upon Acineta papillifera, the intrinsic merits of which have almost entirely been overlooked, owing largely, no doubt, to the fact that it was written in Russian. Keppen, in his paper published in the 'Memoires de la Societé des Naturalistes de la Nouvelle Russie,' Odesse, T. 13, 1888, states that he had not been able to follow the whole process of conjugation, of which he could only find some stages, but that the little he had seen had led him to believe that conjugation in this group, as in the Infusoria, was connected with a reformation of the nucleus, the new macronucleus being formed from the division of the micronucleus. He described in some detail the breaking down of the macronucleus, and in the later stages he saw amongst the degenerating masses of the old macronucleus lightly-staining bodies, which he regarded, on the analogy of Ciliata, as the division products of the micronuclei. Keppen figures four stages in conjugation, of which fig. 50 shows the macronuclei drawn out into long strands and two micronuclear spindles, and fig. 31 shows an Acineta with a new light-staining nucleus surrounded by the small spherical products of the degeneration of the old nucleus.

At the time at which Keppen wrote the part played by the micronucleus in the conjugation of ciliates was not thoroughly understood, but it is to him we owe the first definite recognition of a micronuclear spindle in an acinetarian.

At the date at which Bütschli wrote his account of the Suctoria in 'Bronn's Thierreich' there were numerous isolated references to conjugation amongst Acinetaria to be found in the literature of this group. But as the only observers—Plate and Schneider—(with the exception of Keppen vide supra) who had followed the internal changes connected with this process, denied the existence of a micronucleus, it will be seen that much remained to be done. Bütschli himself remarks, p. 1917:—"Die vorstehenden Erwagungen zeigten, dass Copulationen nicht mit genügenden Sicherheit erwiesen sind. Dagegen ist für die Dendrocometinen sicher, dass ihre conjugation im Wesentlichen wie die partielle der Ciliaten verlauft, woraus wohl geschlossen werden darf, dies gelten auch fur die übrigen, nicht genauer untersuchten Conjugation."

In Maupas' paper, "Le Rajeunissement Karyogamique chez les Cilies" (1889), he mentions without figures the results he had obtained in the conjugation of two acinetarians Podophrya fixa and Podophrya cyclopum. In both these forms there is only a single micronucleus, and in Podophrya cyclopum there is apparently complete fusion without the previous existence of any differentiation between the two gametes.

Maupas describes the conjugation of Podophrya fixa, which, as in the case I describe, is partial, in the following words (p. 385):—"Chez la Podophrya fixa j'ai observé les stades A, B, C and H Pendant ce dernier stade je n'ai jamais vu qu'un seul nouveau corps nucléaire et un seul micronucleus. Je ne serais donc pas étonné que la seconde division de la nucleus mixte de copulation, correspondant au stade G, ne se produise pas chez cette espèce. L'ancien Noyau se desorganise et disparait."

As regards the structure of the micronucleus, Maupas only states (p. 386) that it is "beaucoup plus tenu que chez les Ciliés, et sa substance se colore fort peu par les tinctures microchemiques. De là, avec d'autres causes qu'il serait trop long d'enumerer, ici les grandes difficultés de la mise en evidence. Elles sont, en effet, si grandes, que je considère l'étude d'une de les conjugations d'acinetiens, comme une des recherches les plus pénibles qu'un micrographe puisse entreprendre."

The next account of conjugation in Acinetaria is to be found in Réné Sand's 'Étude Monographique sur le Groupe des Infusoires Tentaculiferes, 1901,' and I do not think that it can be regarded in any way as marking an advance in our knowledge of the process in this group, mainly, apparently, on account of the author's obsession by the theory that the Acinetaria are descended from the Helioza.

Sand considered that the micronucleus is a centrosome, and that the so-called conjugation is a plastogamy with two main aims (p. 101) :

(1) Une rènovation, un rajeunissement.

(2) Un processus de nutrition et de conservation ayant pour but d'égaliser, de'moyenniser' leur situation nutritive.

His figures of the process do not show the nuclei at all, and apparently the whole of the observations, as apart from the conclusions which he formed, are contained in the following sentences (p. 99) :

"Nous avons toujours trouvé les deux individus placés sommet contre sommet, leur axes étant dans le prolongement l'un de l'autre; les tentacules étaient rétractés; les conjugaisons étaient très rares; les deux individus, au stade observé, étaient separés par une membrane, les noyaux étaient fragmentés et les cytoplasmas semblaient appelés a devoir se mélanger malgré la presence d'une membrane qui les separait."

"Il était visible que la conjugaison avait lieu entre un individu riche, et un individu pauvre en tinctine."

In 1902 Hickson and Wadsworth published a detailed account of the conjugation of what was at one time regarded as a very aberrant Suctorian Dendrocometes paradoxus, owing to the peculiar structure of the tentacular arms. They showed that the conjugation in this form was quite analogous to the process which occurred in the Ciliata. They found that there were normally three micronuclei, which undergo two successive divisions. Of the products of these divisions one divided again to form the male and female pronuclei. After cross-fertilisation the cleavage nucleus divides again twice in succession; there appears to be some doubt as to some of the later stages in the formation of the new nuclei, but normally two micronuclei degenerate; one becomes the new micronucleus, and the other develops into the macronucleus. In other cases the new macronucleus is formed by the fusion of two micronuclei, and in still other cases the three micronuclei divide again, the later stages in this form of evolution of the new nuclei not being followed. There are, however, some points in their paper, more especially as regards the part played by the macronucleus during this process, and also their general conclusions on the morphology of the cell body in Infusoria, to which it will be necessary to return after describing the conjugation of Acineta papillifera.

Methods. — As fixatives, Flemming's weak solution, Schaudinn's mixture, and corrosive-acetic were chiefly used, of which the weak Flemming solution seemed most suitable.

The preparations were stained either in alum-carmine or iron hæmatoxylin.

The material fixed in the osmic mixture was treated with picro-carmine before staining in alum-carmine.

Acineta papillifera (Keppen).—This species was first described by Keppen in his paper in the 'Memoires de la Societé des Naturalistes de la Nouvelle Russie Odesse'

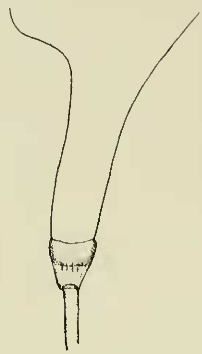
(1888); he separated it from Acineta tuberosa, which it somewhat closely resembles owing to the presence of characteristic "valves" near the junction of the theca with the stalk, and to the fact that the body of the animal is usually not attached to the base of the theca. Keppen found it in both fresh and salt water in the neighbourhood of Odessa.

I first found this acinetarian in June, 1907, growing in great abundance on the hydrocaulus of Cordylophora lacustris, in Hickling Broad, the water of which is slightly brackish. It seemed to be feeding mainly on Stentor, which was at this time very abundant in the broad. As regards the main features of its structure, I have nothing material to add to Keppen's account, except as regards the relation of the theca to the body of the Acineta, and also as regards some details in the process of reproduction.

The "valves."—I always found three of these structures at the junction of the theca and the stalk, presenting under a low power an appearance corresponding with Keppen's figures, but in the interpretation of this appearance I should feel inclined to differ from Keppen.

Keppen regards these structures as three parallel plates cutting off the cavity of the stalk from that of the theca. I think that this appearance is merely the optical expression of a joint consisting of a short tube overlapping the ends of the stalk and of the theca.

These structures are unfortunately very difficult to make out in permanent preparations, but I arrived at this interpretation from an examination of the living animal before I had seen Keppen's paper, and it is, I think, confirmed by longitudinal sections (cf. text-figure 1). This interpretation would explain the frequent occurrence of individuals which have bent laterally at the junction of the stalk and the theca, so that the apical surface faces the base of the stalk. This bending is in some cases, I believe, purely passive, but I have often seen animals turn in this way by wrapping their tentacles around the stalk, and so pulling themselves around. Cases in which either the stalk or the theca itself is bent are much more uncommon.



Text-figure 1.—Section showing relation of theca and stalk in Acineta papillifera.

Cytoplasm.—The body of Acineta papillifera is prolonged anteriorly and laterally into two lobes from which the two bundles of tentacles arise. In the individuals from Hickling broad there were almost always two vacuoles lying side by side. Of these, one appeared to be a true contractile vacuole with a period of about one minute, whereas the other appeared to act as a reservoir, maintaining a constant size about a quarter of that of the full contractile vacuole.

In more or less starved forms the cytoplasm is quite hyaline, but in better fed forms the protoplasm may become very opaque owing to the presence of certain bodies with an affinity for nuclear stains. These bodies are apparently analogous with the Tinctin-körper of Plate VIII, the origin of which I have already dealt with in the case of Tokophrya elongata.

The macronucleus is generally an oval structure lying more or less centrally in the cytoplasm. Generally in whole stained preparations numerous spherical dark areas are to be seen resembling the so-called "Binnen-körper" of the Infusoria. In section these structures, as in the case of some Infusoria (Bütschli, loc. cit., p. 1510) and Dendrocometes (Hickson), are found to consist merely of local thickenings in the mesh of the nuclear network, and therefore resemble karyosomes rather than true nucleoli.

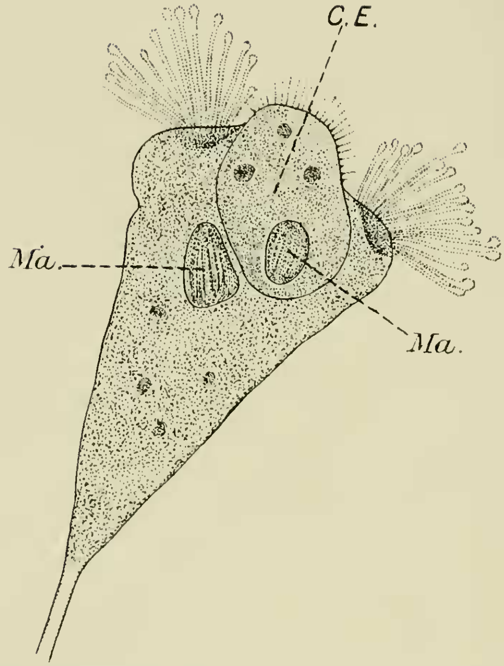
The micronucleus in the resting stage lies in a depression of the macronucleus, and consists of a membrane surrounding a clear area, in the centre of which lies a mass of feebly staining chromatin granules.

Reproduction.—As regards reproduction, the process which Keppen termed external budding is referred to at the end of the section upon conjugation. The only method of reproduction observed in the Acineta papillifera from Hickling was by the formation of single internal ciliated buds.

Keppen describes in addition to this for his form from Odessa, a process of multiple budding, but the figures that he gives are not convincing. One figure seems to me to show quite clearly that one so-called bud is simply a small individual with a fully developed stalk focussed through the large individual. The main features of the formation of the internal bud correspond with Bütschli's description of the formation of the internal buds in Tokophrya quadripartita. A small depression is formed on the apical surface of the acinetarian, which gradually widens so as to cut out a portion anterior to the macronucleus, the future bud.

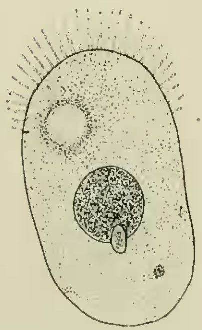
The free bud has a roughly cylindrical but slightly flattened shape (text-figs. 2 and 3). On its physiologically ventral surface it is covered with numerous rows of cilia. (Keppen speaks of 6—11 rows of cilia, but in the form from Hickling broad they seemed more numerous.)

There is a contractile vacuole on the left side near the anterior end, with a small reserve vacuole near it. The oval macronucleus lies near the centre of the body, and in favourable cases a micronucleus can be seen lying in a depression of the nuclear membrane. At first, after its escape, the embryo moves rather slowly, but a little later it moves rapidly through the water in characteristic sinuous curves.

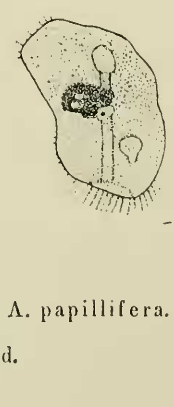


TEXT-FIGURE 2.—Escaping ciliated bud of A. papillifera.
C.E. Ciliated bud. *Ma.* Macronucleus.

TEXT-FIG. 3.



TEXT-FIG. 4.

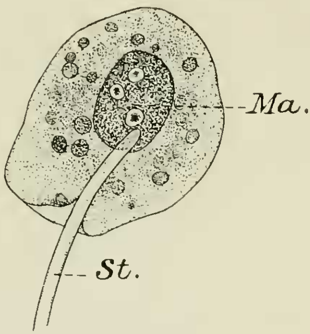


TEXT-FIGURE 3.—Free ciliated bud of A. papillifera. (Zeiss 2 mm., apochr., comp. oc. 6.)

TEXT-FIGURE 4.—Later stage of free bud.

In an embryo (text-fig. 4) which left its mother at 10.45 p.m., the movements became very slow at about midnight, and it was fixed at 2 a.m. At this period the cilia had not yet disappeared, but the commencement of the stalk was already to be seen.

A slightly later stage of the development is seen in text-fig. 5, in which the stalk had almost attained its definitive length, and the cilia have disappeared. At a still later stage the tentacles make their appearance scattered irregularly



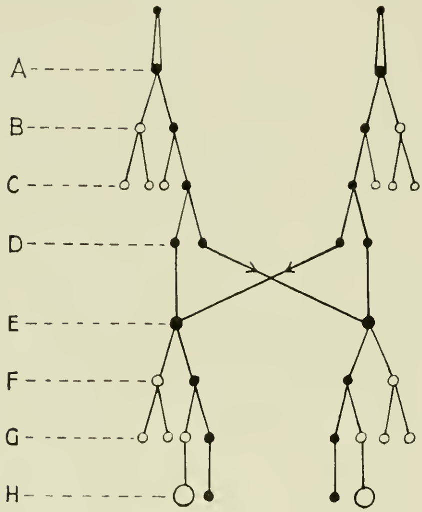
TEXT-FIGURE 5.—A. papillifera soon after attachment, showing disappearance of cilia and development of stalk. (Zeiss 2 mm. apochr., comp. oc. 6.) *Ma.* Macronucleus. *St.* Stalk.

over the surface of the body, and the theca develops as a cup surrounding the body at its junction with the stalk.

Finally, the superfluous tentacles are withdrawn, and the animal becomes compressed laterally, assuming the shape of the fully developed form, the theca growing up to surround the whole body with the exception of a slit at the apical extremity, through which the lateral lobes bearing the tentacles protrude, and the ciliated embryos escape.

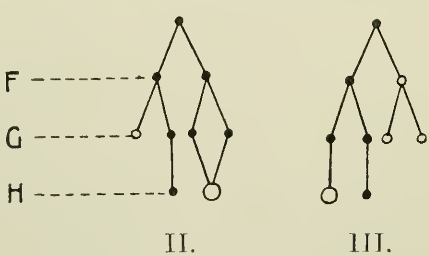
Conjugation.—As regards the early details of conjugation, there is no doubt that they agree with Maupas's general scheme for Ciliates (Scheme I).

In preparations of the later stages the small size of the micronuclei, and the large number of chromatin-masses scattered irregularly throughout the cytoplasm render it difficult to attain to absolute certainty as to the mode of



SCHEME I.—General scheme to illustrate the behaviour of the micronuclei of Ciliates in relation to conjugation—after Maupas. Micronuclei small black dots. Degenerating micronuclei small circles. New macronuclei large circles.

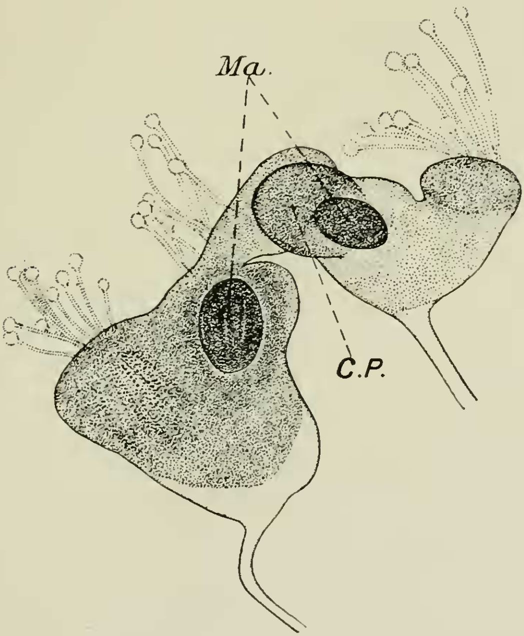
development of the new micro- and macro-nucleus. At first, I believed the normal process of reconstruction involved the



SCHEMES II and III.—Diagrammatic schemes to illustrate the two possible modes of nuclear reconstruction after conjugation in *A. papillifera*.

growth of two of the four micronuclei into macronuclei, and their subsequent fusion (Scheme II). Later, however, I became more inclined to the view that normally one micronucleus develops into the new macronucleus, one remains as the new micronucleus and two degenerate (Scheme III).

It is quite possible that both these methods of reconstruction occur normally since Prandtl has shown recently in a very careful work on conjugation in Didinium nasutum,



TEXT-FIGURE 6.—A. papillifera, first stage of conjugation, showing the prolongations (*C.P.*) by which contact between conjugating individuals is effected. *Ma.* Macronuclei. (Zeiss 2 mm. apochr., comp. oc. 6.)

that here the nuclear apparatus may be restored by one out of no less than five different methods, and Hickson has described three alternative methods in Dendrocometes.

The first indication of conjugation was found by Professor Minchin among specimens collected and examined on August 29th, and the majority of the individuals fixed at this period were found on examination to be early stages A—E. I have no evidence as to the period occupied by the entire process of conjugation, but the pair figured in Pl. VII, fig. 8, which were found on fixation to have reached the final stage in the reconstruction of the nuclei, were followed for forty-six hours, but it is possible that this period is largely influenced by external conditions, e. g. temperature. Contact is usually effected between two conjugants by the prolongation of the apical lobes, the prolongations usually shortening as conjugation proceeds (text-fig. 6). More rarely the apical lobe of one conjugant may come in contact with the side of the other conjugant (Pl. VII, fig. 8), and a still more rare conjugation is purely lateral. In these latter cases the thin wall of the theca of one or both conjugants is dissolved away, so as to allow the contact between the cytoplasm of the two individuals.

Stage A.—The first internal indication of conjugation is afforded by the macronucleus. In Pl. VII, fig. 1, the macronucleus of the individual labelled A still shows the characteristic appearance of the resting nucleus with large darkly-staining areas, the pseudo-nucleoli. The micronucleus is unchanged, and closely applied to the macronucleus. The tentacles are partly withdrawn.

In B the macronucleus has already taken on the coarsely fibrillar appearance which is absolutely characteristic of the stages of its degeneration during conjugation. The micronucleus has left its original position near the macronucleus, and is preparing for the first division.

Stage B (Pl. VII, fig. 2).—The macronuclei of both individuals have become elongated, and the fibrillar appearance is more marked. The dividing micronucleus in the left-hand individual is covered by the macronucleus. The appearance of the micronucleus in the right-hand form seems to present some analogy to the so-called "Sichel" stage in the first division of the micronucleus in conjugating Paramecia described by Hertwig, and more recently by Hamburger.

Stage C.—Both individuals of the conjugation shown in

Pl. VII, fig. 3, contained two micronuclear spindles, which were slightly smaller than those of the first division.

Stage D.—This stage is, I believe, represented by Pl. VII, fig. 4. In each individual there is one micronuclear spindle near the line separating the two individuals, which would give rise to the male and female pronuclei. Of the other micronuclei, two in process of degeneration lie near the contractile vacuole, and the third is probably concealed by the macronucleus.

Stage E.—This stage is shown in Pl. VII, fig. 5, and the details of the male and female pronuclei under a slightly higher power in Pl. VII, fig. 6.

At this stage the degenerating macronuclei have become enormously drawn out, and as their length is so great, they are frequently thrown into coils which may abut on the partition dividing the conjugation, and readily give the appearance of a macronuclear conjugation. It is to such appearances as this that I think Hickson's account of macronuclear conjugation in Dendrocometes paradoxus must be attributed, and as Hickson seems inclined to attach much importance to this process (vide p. 349: "I am prepared, however, to go further than Sand, and regard the presence of the meganuclei in the conjugation processes not only as evidence of their relation to the interchange taking place in the cytoplasm, but as evidence of the necessity of the interchange of molecules of the substance of the meganucleus itself") it will be necessary to examine in some detail the evidence on which this meganuclear conjugation rests. This appears to me to be contained in the two following passages:

> (1) Page 331. "At some time during the last three stages (H, J, K) the old meganucleus becomes very large, and is bent on itself in the form of a loop or horse-shoe. One extremity of this figure passes into the conjugative process, and approaching the limiting membrane, traverses it and fuses with the corresponding extremity of the meganucleus in the other individual. The exact phase at which this meganuclear conjugation takes place seems

to vary considerably; all that can be said at present is that, as far as my experience goes, it usually occurs between stages I and K."

(2) Page 342. "In my preparations of Dendrocometes I have at least three cases in which the meganuclei actually touch, but a considerable number in which they approach one another very closely in the conjugation processes. That the junction is not merely casual contact, but actual organic connection, is proved by the preparation which is represented in Pl. 18, fig. 11. Here there is no sign of any boundary between the two nuclei, and the chromatin granules are fixed in such a manner as to suggest very forcibly that during life they were flowing from one side to another."

As at this stage of conjugation the macronuclei are rapidly degenerating, Hickson, in his attempt to show that this contact of the macronuclei is of fundamental importance in conjugation, puts forward the theory that "there is no inconsistency in the view that after the disappearance of the old megannucleus its nucleoplasm is still living in a modified form diffused through the cytoplasm."

The latter stages in the degeneration of the macronucleus in a conjugating acinetarian are almost precisely similar to the stages figured by Schaudinn in the digestion of the nucleus of a Trichosphærium sieboldi which has been devoured by one of its congeners, and to the stages which I hope to describe in the digestion of the nucleus of Stentor by Tokophrya, and I believe that it is quite as justifiable to speak of the nucleoplasm still living in a modified form diffused through the cytoplasm in the one case as in the other.

I feel inclined, until further evidence is adduced, to regard the appearance figured by Hickson as abnormal, the forces which tend to elongate the macronuclei in the degenerating stages having carried the process too far, and breaking through the partition dividing the two conjugants led to the apparent macronuclear conjugation. In both the conjugants in fig. 5 the remains of three degenerating micronuclei are to be seen.

In Pl. VII, fig. 6, the conjugating processes of the same pair are seen under a higher magnification. In the right-hand form the spindle of the fused male and female pronuclei is already formed. In the left-hand form the female pronucleus is lying close to the partition separating the two conjugants, whilst the male micronucleus is in a depression between the two conjugating forms.

This stage seems to be comparable with that figured by Hickson for Dendrocometes, Pl. 17, fig. 10, and shows that here, as in Dendrocometes, in contradistinction to the state of affairs found in Paramecia, the male and female pronuclei fuse not in the spindle, but in the resting stage.

It is also interesting to find that here, as in the case of conjugation in Didinium nasutum described by Prandtl, the male pronucleus seems to be considerably smaller than the female pronucleus.

The later stages in conjugation become exceedingly difficult to follow, as the chromatin of the macronucleus seems to be dissolved out of the achromatic network, and to be scattered through the cytoplasm in the form of darkly-staining, irregularly spherical blocks.

Normally, the zygote nucleus formed by the fusion of the male and female pronucleus appears to divide twice in succession, and of the products of this division two degenerate —one becoming the new micronucleus, and one the new macronucleus.

In Pl. VII, fig. 7, the achromatic network of the old macronucleus (*Ma.F.*) is still to be seen, though nearly all the chromatin appears to have been dissolved out, and to lie in irregular blocks (*Chr.*) in the cytoplasm.

Two of the division products of the zygote nucleus are to be seen as faintly staining vacuolar bodies, both closely applied to a mass of chromatin (*Mi.*). This relation suggests the absorption of the dissolved chromatin by the growing nucleus, a process to which Hamburger has drawn attention in her recent account of conjugation in Paramecium. In this case probably the new macronucleus would have resulted from the fusion of these two bodies, the new micronucleus being probably covered by some part of the old macronucleus.

I can find no evidence in my preparations in support of Hickson's view that the feeble-staining capacity of the developing macronucleus is due to the fact that "at this stage either the whole or the greater part of the chromatin in its modified form passes into the surrounding cytoplasm, leaving the new meganucleus perfectly clear and homogeneous" (p. 345), a process which Hickson compares to the extrusion of chromatin in the egg cells of some Metazoa. I am more inclined to believe that this primary loss of staining power on the part of the developing macronucleus is to be explained by a simple increase of size, which is compensated for at a later stage by the absorption of the chromatin from the remains of the old macronucleus.

The final stage in the reformation of the nuclei is shown in Pl. VII, fig. 8. This pair of conjugants was examined at intervals from 10.45 p.m. on August 31st to 8.10 p.m. on September 2nd. Unfortunately, nothing could be made of the micronuclear changes in the living animal. During the last five hours the tentacles, which had been previously shortened, commenced to elongate, and when the individuals were killed they had almost reached their normal length.

From other observations on living material I am led to believe that the tentacles are not usually withdrawn during the early period of conjugation up to the stage F or G; during the much longer period associated with the final disappearance of the old macronucleus and the development of the new one the tentacles may become much shortened, and only attain their normal length after the reconstruction of the macronucleus, and shortly before the separation of the conjugants.

In the final stage the macronucleus has a very characteristic appearance of a lightly-staining loose mesh, with scattered chromatin granules, and all traces of the old macronucleus have disappeared (Pl. VII, fig. 8).

External budding.—It will be now necessary to refer to a process which Keppen has termed "external budding," and which he describes as the development of a lateral outgrowth, which may be followed by disappearance of the tentacles. He found that this outgrowth may change its shape and size considerably, and after a period of ten to twelve hours the animal may return to its original shape. In some very rare cases the outgrowth may break off, but he was never able to determine the fate of the bud so formed. In several cases he observed the nucleus carried to the boundary between the acinetarian and the so-called bud, and under these conditions he found appearances of fragmentation of the nucleus, though there was nothing to suggest a normal division.

In one case (fig. 47) he found a specimen of Acineta papillifera with a spade-like body containing a nucleus, and covered with moving cilia attached near the tentacles. This body, owing to its resemblance in shape, he was inclined to compare to the outgrowths described above. The body remained in contact with the acinetarian for some time, until they were finally both lost.

These structures are probably identical with those which Fraipont had previously described as "diverticules générateurs" in acinetan division, and which, according to him, were not to be regarded as external buds, but as structures out of which endogenous ciliated buds were to be developed. This theory was based on a single observation in which he found a ciliated bud in contact with a fixed form.

I believe that Keppen has confounded under this term two quite distinct phenomena—

(1) The formation of long conjugation processes in individuals which by reason of their position cannot come into contact with another individual ripe for conjugation.

(2) Cases of conjugation between a fixed form and a free-swimming ciliated embryo.

(1) It is interesting to notice that both Fraipont and Keppen examined individuals during a conjugation epidemic. During the conjugation epidemic which I had the opportunity of observing I found, especially in material fixed during the commencement of the period, numerous examples of these long processes. The early stages of their formation are analogous to those of the formation of the conjugating processes, as may be seen by a comparison of text-fig. 6 and Pl. VII, fig. 9. I was never able to see an instance in which one of these so-called buds became free, but in several examples from later material I have found evidence of change in the nucleus (Pl. VII, fig. 9).

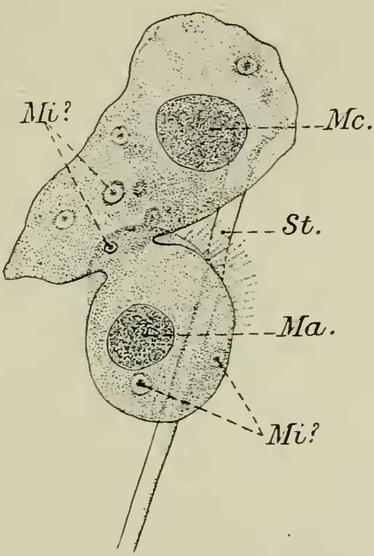
Although I have not been able to follow out these stages in detail, the appearance of the macronucleus is absolutely identical with the fibrillar stage which occurs previous to the fragmentation in normal conjugation.

I am inclined to believe that under certain circumstances, e.g. the absence of mature neighbours within range, a process of parthenogenesis occurs similar to that described by Hertwig for Paramecium aurelia (p. 224), and by means of which Prandtl later has explained the behaviour of the third individual in cases of triple conjugation in Didinium.

It would seem that the chances against two neighbouring acinetaria exhibiting at the same time the three generally accepted symptoms of conjugation, viz. (1) sexual maturity, (2) distant relationship, (3) starvation, would be far more remote than the chance of meeting of two free swimming infusoria. And accordingly it is not surprising that in preparations made during a conjugation epidemic such appearances of presumable parthenogenesis are fairly frequent.

(2) Conjugation between a fixed individual and a free ciliated bud.

It is under this heading that I feel inclined to place Keppen's figure (49). I believe that this process is rare, and, in fact, I was only once able (text-fig. 7) to follow the process in the living individual; but if the cilia, as is the case in the normal bud, disappear at the end of a couple of hours, it might be very difficult to distinguish the later stages of process (2) from those of process (1) (text-figs. 6 and 7).



TEXT-FIGURE 7.—A. papillifera, conjugation between a free ciliated bud and a fixed form. *Ma.*, *Mc.* Macronucleus. *Mi.* ? Micronucleus. *St.* Stalk. (Zeiss 2 mm. apochr., comp. oc. 6.)

IV. CONCLUSIONS.

(1) The Tinctin-körper of Acinetaria are generally fragments of the ingested nucleus of their prey.

(2) Conjugation in Acineta papillifera agrees in all essentials with the process occurring in the Ciliate Infusoria, and that described by Hickson for Dendrocometes paradoxus.

It is possible that in those cases in which a fixed form cannot come into contact with another mature individual, a reorganisation of the nuclei may be effected associated with either (*a*) conjugation with a free swimming ciliate bud, or (*b*) a process of parthenogenesis associated with the formation of the so-called "external buds" of Keppen.

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